Design, Synthesis and Biological Evaluation of C-8 Modified Curcumol Derivatives as Potential Anticancer Agents

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Abstract

A series of structural modification of curcumol derivatives at C-8 position were designed and synthesized, which structures were confirmed by $^1$H NMR, $^{13}$C NMR and HRMS analysis. The tested compounds were evaluated for in vitro antitumor activity against colorectal cancer cell lines SW620, HCT116 and CaCo2. Many of the tested candidates exhibited higher inhibition efficiency than curcumol. Among them, compound 3l shows the best inhibitory effect on the viability of SW620 with IC$_{50}$ value of 19.90 mM. The structure-activity relationships (SARs) of these derivatives were discussed, which showed that the introduction of amino or aryl groups tended to increase the anti-cancer activity. In addition, compound 3l may inhibit cancer cell proliferation through triggering cell apoptosis.

Introduction

Colorectal cancer (CRC) is considered one of the most predominant and deadly cancer globally [1]. Currently, radiotherapy, chemotherapy and surgery are three main therapeutic methods for human colorectal cancer. At present, chemotherapy is still the main type of cancer treatment. First line chemotherapeutic drugs like 5-fluorouracil [2], leucovorin [3], oxaliplatin [4], irinotecan [5], and trifluridine [6] are widely used for the treatment of CRC. However, chemotherapy treatment of CRC is not satisfactory in the clinic because of the toxic side effects. Indeed, natural drugs have been a crucial part of anti-cancer drugs, and more than half of the approved anticancer drugs are either natural products or developed on the basis of knowledge gained from natural products [7–8]. Therefore, one feasible approach of seeking new chemotherapy drug candidate from phytochemicals is to modify the current clinically available anti-CRC medicines. To date, a variety of guaiane-type natural products have been isolated and tested for antitumor activity, such as curcumol [9–10], englerin A [11] and arglabin [12], showed good antitumor efficacy and entered clinical studies (Fig. 1).

Curcumol, is known to possess numerous pharmacological activities against many cancers like lung [13], breast [14] nasopharyngeal [15], gastric [16], colorectal [17], and ovarian carcinoma's [18]. In the long history of the advancement of traditional Chinese medicine, curcumol has played an important role in cancer treatment [19]. Curcumol exhibits synergistic cytotoxic effects on NSCLC via interacting with NQO2 to activate ROS-CHOP-DR5 signaling [20]. A recent report showed that curcumol induces cell cycle arrest in the G1/S phase in human CRC cell lines, LoVo and SW480. Furthermore, the underlying mechanism shows that curcumol could inhibit CRC from proliferating through suppression of the PI3K/Akt pathways. In addition to its efficacy in controlling cell cycle progression, cell survival pathways, curcumol also inhibits the growth of human CRC cells by targeting miR-21 [21]. Yu’s group revealed that curcumol inhibits viability, migration and invasion by regulating miR30a-5p expression and activating the Hippo signaling pathway in CRC cells [22]. These results have shown the utility of curcumol as an anti-proliferative agent in colorectal cancer. However, its clinical development has been challenged by its insolubility and low bioavailability due to its lack of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). Studies on discovering the structure-activity relationship of curcumol revealed that C-8 position and C-14 position are very important in improving the antitumor activity of curcumol [23–24].
The amide group has gained significant attention due to its unique ability to form relevant hydrogen bonding interactions [25–26]. Herein, we designed a series of novel curcumol derivatives by introducing various hydrophilic chains on C-8 position via amidation, and endeavored to improve the anti-cancer activity by comparison with curcumol (Scheme 1).

**Experimental/material And Methods**

**General information**

All the reagents were used without further purification unless otherwise specified. Solvents were dried and redistilled prior to use in the usual manner. All starting materials were commercially available. For product purification by flash column chromatography, silica gel (200~300 mesh) was used. The reactions monitoring was accomplished by TLC on silica gel polygram SILG/UV 254 plates. $^1$H NMR spectra were recorded at 400 MHz or 500 MHz in CDCl$_3$ using TMS as internal standard. $^{13}$C NMR spectral measurements were performed at 125 MHz or 101MHz using TMS as an internal standard. All compounds were identified by $^1$H NMR and are in good agreement with those reported. HRMS(ESI) was determined on a PerkinElmer spectrometer.

1. **General procedure for the preparation of compounds 2a-2k**

To a solution of various secondary amine (1.0 eq.) in 20 mL of DCM was added Et$_3$N (2.0 eq.) and chloroacetyl chloride (1.0 eq.) in an ice bath under N$_2$. The reaction mixture was allowed to warm to room temperature and continued to be stirred for 3-5 h. The reaction was monitored by TLC. After completion, the reaction was quenched by addition of water and extracted with EtOAc (3×20 mL). The organic phase was dried over anhydrous Na$_2$SO$_4$. After condensation under reduced pressure, the obtained compounds 2a-2k were directly used into the next step without further purification.

2. **General procedure for the preparation of compounds 3a-3k**

To a solution of curcumol (1.0 eq.) in 25 mL dry THF, NaH (5.0 eq.) was added and the reaction mixture was stirred at room temperature for 2 h. Then, the compounds 2a-2k (1.0 eq.) was added at room temperature for 5-8 h. Reaction was monitored by TLC. The reaction is quenched by water and extracted with EtOAc (3 × 20 ml). The combined organic layer was dried over Na$_2$SO$_4$ and purified through column chromatography to offer the corresponding products.

2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)-N,N-dimethylacetamide (3a): Yellow oil (395 mg, 58% yield), $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.77 (s, 2H), 4.29 (d, $J = 12.5$ Hz, 1H), 4.11 (d, $J = 12.5$ Hz, 1H), 3.01 (s, 3H), 2.85 (s, 3H), 2.50 (d, $J = 14.7$ Hz, 1H), 2.40 (d, $J = 14.7$ Hz, 1H), 2.12-2.05 (m, 1H), 2.02-1.94 (m, 1H), 1.90-1.75 (m, 2H), 1.75-1.66 (m, 1H), 1.66-1.50 (m, 3H), 1.31-1.38 (m, 1H), 1.08 (dd, $J = 12.5$, 6.4 Hz, 1H), 0.87 (t, $J = 6.2$ Hz, 6H), 0.76 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 169.24, 144.54, 112.61, 107.26, 87.49, 62.03, 54.36, 49.63, 39.16, 37.10, 36.70, 35.24,
33.97, 30.81, 28.36, 27.97, 22.71, 20.74, 12.04; HRMS m/z (ESI): calcd for C_{19}H_{32}NO_3 [M+H]^+: 322.2377, found: 322.2379.

\textbf{N,N-diethyl-2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)acetamide (3b):} Yellow oil (385 mg, 52% yield), \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \delta 4.83 (s, 2H), 4.34 (d, J = 12.1 Hz, 1H), 4.14 (s, 1H), 3.38-3.48 (m, 2H), 3.28-3.37 (m, 2H), 2.55 (d, J = 15.7 Hz, 1H), 2.46 (d, J = 14.8 Hz, 1H), 2.18-2.10 (m, 1H), 2.04 (t, J = 12.3 Hz, 1H), 1.97-1.80 (m, 2H), 1.81-1.72 (m, 1H), 1.71-1.57 (m, 3H), 1.37-1.44 (m, 1H), 0.94 (d, J = 6.5 Hz, 6H), 0.82 (d, J = 6.4 Hz, 3H); \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \delta 168.64, 144.87, 112.78, 107.52, 87.61, 62.33, 54.62, 49.68, 41.30, 39.90, 39.39, 37.49, 34.19, 31.03, 28.61, 28.22, 22.92, 21.00, 14.27, 12.69, 12.24; HRMS m/z (ESI): calcd for C_{21}H_{36}NO_3 [M+H]^+: 350.2693, found: 350.2690.

\textbf{N,N-dibutyl-2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)acetamide (3c):} Yellow oil (515 mg, 60% yield), \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \delta 4.78 (s, 2H), 4.31 (d, J = 12.1 Hz, 1H), 4.08 (d, J = 12.1 Hz, 1H), 3.36-3.11 (m, 4H), 2.53-2.37 (m, 2H), 2.11 (d, J = 10.3 Hz, 1H), 1.99 (t, J = 12.3 Hz, 1H), 1.91-1.76 (m, 2H), 1.69-1.75 (m, 1H), 1.67-1.55 (m, 3H), 1.42-1.51 (m, 4H), 1.41-1.32 (m, 1H), 1.20-1.29 (m, 5H), 1.10 (dd, J = 12.5, 6.4 Hz, 1H), 0.90 (d, J = 6.6 Hz, 6H), 0.83-0.87 (m, 6H), 0.78 (d, J = 6.3 Hz, 3H); \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \delta 168.76, 144.74, 112.66, 107.38, 87.50, 62.08, 54.54, 49.51, 46.91, 45.27, 39.30, 37.42, 34.12, 30.98, 30.89, 29.42, 28.50, 28.11, 22.82, 20.91, 20.06, 19.89, 13.70, 13.67, 12.1; HRMS m/z (ESI): calcd for C_{25}H_{44}NO_3 [M+H]^+: 406.3316, found: 406.3315.

\textbf{2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)-1-(pyrrolidin-1-yl)ethan-1-one (3d):} Yellow oil (391 mg, 53% yield), \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \delta 4.82 (s, 2H), 4.30 (d, J = 12.9 Hz, 1H), 4.09 (d, J = 12.9 Hz, 1H), 3.52 (s, 2H), 2.53-2.37 (m, 2H), 2.11 (d, J = 10.3 Hz, 1H), 1.99 (t, J = 12.3 Hz, 1H), 1.91-1.76 (m, 2H), 1.69-1.75 (m, 1H), 1.67-1.55 (m, 3H), 1.42-1.51 (m, 4H), 1.41-1.32 (m, 1H), 1.45-1.33 (m, 1H), 0.93 (d, J = 6.5 Hz, 6H), 0.83-0.87 (m, 6H), 0.78 (d, J = 6.3 Hz, 3H); \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \delta 167.48, 144.74, 112.69, 107.37, 87.55, 62.58, 54.50, 49.51, 46.91, 45.27, 39.30, 37.42, 34.12, 30.98, 30.89, 29.42, 28.50, 28.11, 22.82, 20.91, 20.06, 19.89, 13.70, 13.67, 12.1; HRMS m/z (ESI): calcd for C_{21}H_{34}NO_3 [M+H]^+: 348.2533, found: 348.2531.

\textbf{2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)-1-(piperidin-1-yl)ethan-1-one (3e):} Yellow oil (490 mg, 64% yield), \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \delta 4.77 (s, 2H), 4.27 (d, J = 12.0 Hz, 1H), 4.09 (d, J = 12.0 Hz, 1H), 3.37-3.52 (m, 4H), 2.48 (d, J = 14.7 Hz, 1H), 2.39 (d, J = 14.7 Hz, 1H), 2.08 (t, J = 10.0 Hz, 1H), 1.98 (t, J = 12.3 Hz, 1H), 1.90-1.75 (m, 2H), 1.66-1.73 (m, 1H), 1.56-1.63 (m, 6H), 1.52-1.42 (m, 5H), 1.34 (m, 1H), 1.08 (dd, J = 12.4, 6.4 Hz, 1H), 0.93-0.83 (m, 6H), 0.76 (d, J = 6.4 Hz, 3H); \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \delta 167.48, 144.48, 112.56, 107.20, 87.38, 62.18, 54.33, 49.44, 46.13, 42.65, 39.13, 37.18, 33.90, 30.79, 28.33, 27.95, 26.09, 25.25, 24.30, 22.66, 20.78, 12.00; HRMS m/z (ESI): calcd for C_{22}H_{36}NO_3 [M+H]^+: 362.2690, found: 362.2689.
1-(azepan-1-yl)-2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)ethan-1-one (3f): Yellow oil (548 mg, 69% yield), $^1$H NMR (500 MHz, CDCl$_3$) δ 4.76 (s, 2H), 4.29 (d, $J = 12.0$ Hz, 1H), 4.07 (d, $J = 12.0$ Hz, 1H), 3.51-3.36 (m, 4H), 2.47 (d, $J = 14.2$ Hz, 1H), 2.39 (d, $J = 14.7$ Hz, 1H), 2.12-2.03 (m, 1H), 1.97 (t, $J = 12.3$ Hz, 1H), 1.74-1.80 (m, 2H), 1.61-1.72 (m, 9H), 1.47 (s, 5H), 1.38-1.27 (m, 1H), 1.07 (dd, $J = 12.5$, 6.4 Hz, 1H), 0.87 (dd, $J = 6.5$, 2.4 Hz, 6H), 0.75 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 168.66, 144.50, 112.53, 107.20, 87.30, 62.04, 54.32, 49.38, 46.93, 46.10, 39.10, 37.17, 33.89, 30.77, 29.31, 28.32, 27.92, 27.22, 26.70, 26.29, 22.66, 20.74, 11.99; HRMS m/z (ESI): calcd for C$_{23}$H$_{38}$NO$_3$ [M+H]$^+$: 376.2846, found: 376.2847.

2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)-1-morpholinoethan-1-one (3g): Yellow oil (385 mg, 50% yield), $^1$H NMR (500 MHz, CDCl$_3$) δ 4.78 (s, 2H), 4.27 (d, $J = 12.2$ Hz, 1H), 4.11 (d, $J = 12.2$ Hz, 1H), 3.66-3.55 (m, 7H), 3.51 (d, $J = 4.4$ Hz, 2H), 2.33-2.52 (m, 2H), 2.09 (t, $J = 10.1$ Hz, 1H), 1.99 (t, $J = 12.3$ Hz, 1H), 1.92-1.74 (m, 2H), 1.66-1.72 (m, 1H), 1.65-1.53 (m, 3H), 1.29-1.36 (m, 1H), 1.09 (dd, $J = 12.5$, 6.4 Hz, 1H), 0.86 (dd, $J = 6.4$, 3.6 Hz, 6H), 0.77 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 167.87, 144.17, 112.83, 107.32, 87.61, 66.53, 66.45, 62.07, 54.26, 49.42, 45.76, 42.02, 39.09, 37.15, 33.83, 30.80, 28.30, 27.92, 22.65, 20.82, 12.07; HRMS m/z (ESI): calcd for C$_{21}$H$_{34}$NO$_4$ [M+H]$^+$: 364.2482, found: 364.2481.

1-(3,4-dihydroisoquinolin-2(1H)-yl)-2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)ethan-1-one (3h): Brown oil (347 mg, 40% yield), $^1$H NMR (500 MHz, CDCl$_3$) δ 6.95-7.15 (m, 4H), 4.82 (s, 2H), 4.76 (s, 1H), 4.65 (s, 1H), 4.47-4.38 (m, 1H), 4.20 (dd, $J = 18.2$, 12.2 Hz, 1H), 3.62-3.90 (m, 2H), 2.85 (t, $J = 5.3$ Hz, 1H), 2.79 (s, 1H), 2.63-2.35 (m, 2H), 2.13 (t, $J = 9.9$ Hz, 1H), 2.09-1.99 (m, 1H), 1.97-1.73 (m, 4H), 1.55-1.71 (m, 4H), 1.13 (dd, $J = 12.4$, 6.3 Hz, 1H), 0.89-0.92 (m, 6H), 0.81 (t, $J = 5.7$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 168.22, 144.23, 133.87, 132.68, 128.09, 126.16, 112.73, 107.28, 87.45, 62.36, 54.23, 49.17, 46.82, 44.19, 42.60, 39.92, 39.03, 37.13, 33.81, 30.81, 29.12, 28.27, 27.90, 22.64, 20.69, 12.04 ppm; HRMS m/z (ESI): calcd for C$_{26}$H$_{36}$NO$_3$ [M+H]$^+$: 410.2690, found: 410.2692.

2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)-1-(4-phenylpiperazin-1-yl)ethan-1-one (3i): Yellow oil (724 mg, 78% yield), $^1$H NMR (500 MHz, CDCl$_3$) δ 7.34-7.21 (m, 2H), 7.02-6.80 (m, 3H), 4.89 (s, 2H), 4.43 (d, $J = 12.2$ Hz, 1H), 4.25 (d, $J = 12.2$ Hz, 1H), 3.73-3.90 (m, 4H), 3.29-3.05 (m, 4H), 2.58 (d, $J = 14.8$ Hz, 1H), 2.51 (d, $J = 14.7$ Hz, 1H), 2.23-2.16 (m, 1H), 2.10 (t, $J = 12.3$ Hz, 1H), 2.02-1.86 (m, 3H), 1.79-1.84 (m, 1H), 1.75-1.64 (m, 3H), 1.50-1.40 (m, 1H), 1.19 (dd, $J = 12.5$, 6.5 Hz, 1H), 0.98 (dd, $J = 11.8$, 6.5 Hz, 6H), 0.87 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 167.82, 150.83, 144.29, 128.89 (Ar-overlap), 120.06, 116.33(Ar-overlap), 112.88, 107.38, 87.63, 62.24, 54.31, 49.61, 49.41, 48.93, 45.06, 41.58, 39.15, 37.29, 33.90, 30.89, 28.38, 28.02, 22.75, 20.94, 12.20 ppm. HRMS m/z (ESI): calcd for C$_{27}$H$_{39}$N$_2$O$_3$ [M+H]$^+$: 439.2955, found: 439.2957.
MHz, CDCl$_3$ δ 4.78 (s, 2H), 4.34-4.21 (m, 1H), 4.21-4.01 (m, 1H), 3.60-3.20 (m, 4H), 3.03 (s, 2H), 2.87 (s, 1H), 2.81 (s, 3H), 2.55-2.33 (m, 2H), 2.10 (t, $J = 9.9$ Hz, 1H), 1.92-2.05 (m, 1H), 1.77-1.86 (m, 2H), 1.57-1.63 (m, 4H), 1.38 (s, 9H), 1.09 (dd, $J = 12.6$, 6.2 Hz, 1H), 0.88 (d, $J = 6.3$ Hz, 6H), 0.77 (d, $J = 5.7$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 169.33, 155.67, 144.56, 112.78, 107.32, 87.59, 61.78, 54.45, 49.69, 45.36, 39.23 (overlap), 37.40, 37.24, 34.70, 34.05, 30.86 (overlap), 28.44, 28.20 (overlap), 28.06, 22.81, 20.92, 12.13; HRMS m/z (ESI): calcd for C$_{26}$H$_{43}$N$_2$O$_5$ [M+H]$^+$: 465.3323, found: 465.3324.

tert-butyl4-(2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)acetyl)piperazine-1-carboxylate (3k): Yellow solid (617mg, 63% yield), m.p. 118-120 °C; $^1$H NMR (500 MHz, CDCl$_3$) δ 4.83 (s, 2H), 4.33 (d, $J = 12.0$ Hz, 1H), 4.16 (d, $J = 12.0$ Hz, 1H), 3.76-3.18 (m, 8H), 2.55-2.33 (m, 2H), 2.08-2.17 (m, 1H), 2.08-1.98 (m, 1H), 1.95-1.78 (m, 2H), 1.69-1.75 (m, 1H), 1.61-1.67 (m, 3H), 1.43 (s, 9H), 1.13 (dd, $J = 12.5$, 6.4 Hz, 1H), 0.91 (t, $J = 6.5$ Hz, 6H), 0.81 (d, $J = 6.3$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 168.31, 154.62, 144.48, 113.14, 107.64, 87.94, 80.14, 62.52, 54.56 (overlap), 45.32, 41.82, 39.38 (overlap), 37.47, 34.13, 31.08, 28.60, 28.35 (overlap), 28.23, 22.93, 21.11 (overlap), 12.38; HRMS m/z (ESI): calcd for C$_{26}$H$_{43}$N$_2$O$_5$ [M+H]$^+$: 463.3166, found: 463.3168.

**General procedure for the preparation of compounds 3l-3m**

To a solution of compound 3j (1.0 eq.) in DCM (10 mL), TFA (1 mL) as added at 0°C and the mixture was stirred for 1 h at 0°C. Reaction was monitored by TLC. Saturated aqueous Na$_2$CO$_3$ was added and the mixture was extracted with DCM. The combined organic layer was dried over Na$_2$SO$_4$ and purified by silica gel column chromatography to provide the corresponding products.

2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)-N-methyl-N-(2-(methylamino)ethyl)acetamide (3l): Yellow oil (168 mg, 71% yield), $^1$H NMR (500 MHz, CDCl$_3$) δ 4.80 (s, 2H), 4.36 (dd, $J = 26.5$, 12.3 Hz, 1H), 4.10-4.17 (m, 1H), 3.56-3.37 (m, 2H), 3.04 (d, $J = 10.3$ Hz, 2H), 2.88 (d, $J = 3.6$ Hz, 2H), 2.74 (t, $J = 6.6$ Hz, 2H), 2.40 (s, 3H), 2.17-2.07 (m, 1H), 2.01 (t, $J = 12.3$ Hz, 1H), 1.92-1.68 (m, 4H), 1.59-1.66 (m, 2H), 1.35-1.41 (m, 1H), 1.09-1.14 (m, 1H), 0.93-0.87 (m, 6H), 0.79 (d, $J = 6.3$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 169.81, 144.55, 112.74, 107.34, 87.60, 54.40, 49.67, 49.16, 48.52, 47.16, 39.19, 37.23, 35.64, 35.37, 34.01, 30.83, 28.41, 28.02, 22.77, 20.89, 12.11; HRMS m/z (ESI): calcd for C$_{21}$H$_{37}$N$_2$O$_3$ [M+H]$^+$: 365.2799, found: 363.2797.

2-(((3S,3aS,5R,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)-1-(piperazin-1-yl)ethan-1-one (3m): Yellow oil (179 mg, 76% yield), $^1$H NMR (400 MHz, CDCl$_3$) δ 4.87 (d, $J = 2.4$ Hz, 2H), 4.37 (d, $J = 12.1$ Hz, 1H), 4.20 (d, $J = 12.1$ Hz, 1H), 3.79-3.54 (m, 4H), 3.09 (s, 2H), 2.80-2.95 (m, 4H), 2.59-2.41 (m, 2H), 2.18 (t, $J = 10.0$ Hz, 1H), 2.08 (t, $J = 12.2$ Hz, 1H), 2.01-1.83 (m, 2H), 1.83-1.73 (m, 1H), 1.73-1.62 (m, 3H), 1.47-1.37 (m, 1H), 1.17 (dd, $J = 12.5$, 6.3 Hz, 1H), 0.95 (dd, $J = 6.4$, 3.4 Hz, 6H), 0.86 (d, $J = 6.3$ Hz, 3H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 168.18, 144.63, 113.12, 107.63, 87.90, 62.42, 54.58,
49.66, 46.36, 46.05, 45.56, 42.63, 39.41, 37.52, 34.16, 31.10, 28.65, 28.26, 22.99, 21.16, 12.41; HRMS m/z (ESI): calcd for C_{21}H_{35}N_{2}O_{3} [M+H]^+: 363.2642, found: 363.2640.

**General procedure for the preparation of compound 4a-4g**

To a solution of compound 3 (1.0 eq.) in 30 mL DCM and placed in an ice bath, and acyl chloride (1.0 eq.) was introduced slowly. The reaction was allowed to come to RT and stirred 2-5 h. Reaction was monitored by TLC. DCM was removed via rotary evaporation. The remaining oil was dissolved in EtOAc and washed with distilled water and the crude mixture was extracted with EtOAc (3×20 mL). The organic phase was dried over anhydrous Na_{2}SO_{4}. After filtration, EtOAc was removed by rotary evaporation and purified through column chromatography to offer the corresponding products.

**1-(4-acetylpiperazin-1-yl)-2-(((3S,3aS,5S,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yloxy)ethan-1-one (4a):** Yellow oil (295 mg, 83% yield), \(^1^H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.76 (s, 2H), 4.28 (d, \(J = 12.3\) Hz, 1H), 4.10 (dd, \(J = 12.4, 5.3\) Hz, 1H), 3.47-3.61 (m, 6H), 3.42-3.32 (m, 2H), 2.41 (t, \(J = 15.4\) Hz, 2H), 2.07 (t, \(J = 10.3\) Hz, 1H), 2.02 (s, 3H), 1.98-1.89 (m, 1H), 1.89-1.71 (m, 2H), 1.71-1.63 (m, 1H), 1.57 (dd, \(J = 12.5, 5.4\) Hz, 3H), 1.38-1.25 (m, 1H), 1.07 (dd, \(J = 12.6, 6.2\) Hz, 1H), 0.83 (t, \(J = 7.5\) Hz, 6H), 0.74 (d, \(J = 6.2\) Hz, 3H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 168.98, 168.09, 143.95, 112.90, 107.27, 87.64, 62.00, 54.15, 49.33, 45.59, 44.83, 41.65, 41.34, 38.99, 37.08, 33.73, 30.75, 28.21, 27.87, 22.59, 20.94, 20.76, 12.07; HRMS m/z (ESI): calcd for C\(_{23}\)H\(_{37}\)N\(_2\)O\(_4\) [M+H]^+: 405.2748, found: 405.2748.

**3-chloro-1-(4-(2-(((3S,3aS,5S,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yloxy)acetyl)piperazin-1-yl)propan-1-one (4b):** Yellow oil (284 mg, 68% yield), \(^1^H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 4.85 (s, 2H), 4.43-4.33 (m, 1H), 4.19 (d, \(J = 12.0\) Hz, 1H), 3.82 (t, \(J = 6.9\) Hz, 2H), 3.59-3.73 (m, 6H), 3.40-3.53 (m, 2H), 2.82 (t, \(J = 6.9\) Hz, 2H), 2.47 (dd, \(J = 17.3, 12.0\) Hz, 2H), 2.16 (dd, \(J = 12.7, 7.5\) Hz, 1H), 2.11-2.00 (m, 1H), 1.83-1.96 (m, 2H), 1.82-1.58 (m, 4H), 1.48-1.32 (m, 1H), 1.16 (dd, \(J = 12.4, 6.1\) Hz, 1H), 0.92 (t, \(J = 6.6\) Hz, 6H), 0.83 (d, \(J = 6.2\) Hz, 3H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 168.39, 168.20, 144.23, 113.18, 112.90, 107.27, 87.64, 62.00, 54.15, 49.33, 45.59, 44.83, 41.65, 41.34, 38.99, 37.08, 33.73, 30.75, 28.21, 27.87, 22.59, 20.94, 20.76, 12.07; HRMS m/z (ESI): calcd for C\(_{24}\)H\(_{37}\)ClN\(_2\)O\(_4\)Na [M+Na]^+: 475.2334, found: 475.2334.

**1-(4-(2-(((3S,3aS,5S,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yloxy)acetyl)piperazin-1-yl)prop-2-en-1-one (4c):** Yellow oil (316 mg, 82% yield), \(^1^H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 6.48 (dd, \(J = 16.8, 10.6\) Hz, 1H), 6.36-6.04 (m, 1H), 5.61-5.64 (m, 1H), 4.76 (s, 2H), 4.28 (d, \(J = 12.3\) Hz, 1H), 4.13-4.06 (m, 1H), 3.75-3.36 (m, 8H), 2.49-2.30 (m, 2H), 2.12-2.04 (m, 1H), 2.01-1.92 (m, 1H), 1.75-1.85 (m, 2H), 1.63-1.68 (m, 1H), 1.62-1.51 (m, 3H), 1.40-1.24 (m, 1H), 1.07 (dd, \(J = 12.4, 6.2\) Hz, 1H), 0.84 (t, \(J = 6.7\) Hz, 6H), 0.75 (d, \(J = 6.3\) Hz, 3H); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 167.99, 165.16, 143.92, 128.00, 126.84, 112.89, 107.28, 87.63, 62.05, 54.13, 53.06, 49.31, 45.09, 41.78, 38.97, 37.09, 33.72, 30.74, 28.19, 27.85, 22.57, 20.75 (overlap), 12.05; HRMS m/z (ESI): calcd for C\(_{24}\)H\(_{36}\)N\(_2\)O\(_4\)Na [M+Na]^+: 439.2567, found: 439.2568.
2-(((3S,3aS,5S,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)-1-(4-pyrimidin-2-yl)piperazin-1-yl)ethan-1-one (4d): Yellow oil (333 mg, 86% yield), $^1$H NMR (500 MHz, CDCl$_3$) δ 8.29 (dd, $J = 4.7$, 1.1 Hz, 2H), 6.49 (t, $J = 4.6$ Hz, 1H), 4.83 (s, 2H), 4.39 (d, $J = 12.0$ Hz, 1H), 4.20 (d, $J = 12.0$ Hz, 1H), 3.95-3.49 (m, 9H), 2.57-2.40 (m, 2H), 2.19-2.11 (m, 1H), 2.05 (t, $J = 12.3$ Hz, 1H), 1.96-1.72 (m, 3H), 1.72-1.57 (m, 3H), 1.46-1.36 (m, 1H), 1.14 (dd, $J = 12.5$, 6.5 Hz, 1H), 0.93 (dd, $J = 11.1$, 6.6 Hz, 6H), 0.82 (d, $J = 6.3$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 168.24, 161.41, 157.60 (Ar-overlap), 144.43, 113.00, 110.16, 107.51, 87.80, 62.40, 49.47, 45.18, 43.85, 43.27, 41.77, 39.30, 37.45, 34.04, 30.99, 28.51, 28.16, 22.85, 21.03, 12.31; HRMS m/z (ESI): calcd for C$_{25}$H$_{36}$N$_4$O$_3$SNa [M+Na]$^+$: 463.2680, found: 463.2681.

1-(4-(2-hydroxyethyl)piperazin-1-yl)-2-(((3S,3aS,5S,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)ethan-1-one (4e): Yellow oil (243mg, 68% yield), $^1$H NMR (500 MHz, CDCl$_3$) δ 4.79 (s, 2H), 4.29 (dd, $J = 12.0$, 2.9 Hz, 1H), 4.13-4.04 (m, 1H), 3.71-3.47 (m, 8H), 3.18 (s, 2H), 2.43 (d, $J = 13.1$ Hz, 5H), 2.10 (t, $J = 10.0$ Hz, 1H), 2.00 (t, $J = 12.3$ Hz, 1H), 1.74-1.92 (m, 2H), 1.74-1.54 (m, 4H), 1.41-1.29 (m, 2H), 1.10 (dd, $J = 12.5$, 6.3 Hz, 1H), 0.87 (t, $J = 5.6$ Hz, 6H), 0.78 (d, $J = 6.4$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 167.92, 144.34, 112.90, 107.37, 87.65, 62.06, 59.36, 57.73, 54.33, 52.97, 52.43, 49.32, 45.10, 41.62, 39.16, 37.33, 33.90, 30.86, 28.38, 28.02, 22.74, 20.91, 12.17; HRMS m/z (ESI): calcd for C$_{23}$H$_{39}$N$_2$O$_4$ [M+H]$^+$: 407.2904, found: 407.2906.

4-(2-(((3S,3aS,5S,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)acetyl)-1,1-dimethylpiperazin-1-ium (4f): Yellow oil (279 mg, 72% yield), $^1$H NMR (500 MHz, CDCl$_3$) δ 4.87 (s, 2H), 4.39 (d, $J = 12.2$ Hz, 1H), 4.18 (d, $J = 12.2$ Hz, 1H), 3.92-3.64 (m, 4H), 3.33-3.12 (m, 4H), 2.78 (s, 3H), 2.47 (s, 2H), 2.20-2.14 (m, 1H), 2.06 (t, $J = 12.0$ Hz, 1H), 1.98-1.83 (m, 2H), 1.78-1.61 (m, 4H), 1.19-1.13 (m, 1H), 0.92 (t, $J = 6.6$ Hz, 6H), 0.84 (d, $J = 6.3$ Hz, 3H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 168.62, 144.05, 113.35, 107.67, 88.01, 62.14, 54.41, 49.26, 45.90, 45.42, 45.12, 41.56, 39.25, 37.54, 34.65, 33.95, 31.03, 28.48, 28.15, 22.82, 20.98, 12.35; HRMS m/z (ESI): calcd for C$_{22}$H$_{37}$N$_2$O$_5$S [M+H]$^+$: 441.2418, found: 441.2417.

2-(((3S,3aS,5S,6R,8aS)-5-isopropyl-3-methyl-8-methyleneoctahydro-6H-3a,6-epoxyazulen-6-yl)oxy)-1-(4-tosylpiperazin-1-yl)ethan-1-one (4g): Yellow oil (383 mg, 81% yield), $^1$H NMR (500 MHz, CDCl$_3$) δ 7.62 (d, $J = 8.3$ Hz, 2H), 7.33 (d, $J = 8.1$ Hz, 2H), 4.86 (dd, $J = 7.2$, 1.9 Hz, 2H), 4.29 (d, $J = 12.2$ Hz, 1H), 4.10 (d, $J = 12.2$ Hz, 1H), 3.84-3.57 (m, 4H), 3.11-2.86 (m, 4H), 2.43 (s, 3H), 2.39 (s, 1H), 2.15 (dd, $J = 11.5$, 8.7 Hz, 1H), 2.03 (t, $J = 12.2$ Hz, 1H), 1.94-1.78 (m, 2H), 1.73-1.49 (m, 4H), 1.27 (d, $J = 14.5$ Hz, 3H), 1.14 (dd, $J = 12.6$, 6.1 Hz, 1H), 0.89 (d, $J = 6.5$ Hz, 3H), 0.82 (d, $J = 6.0$ Hz, 6H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 168.16, 144.29, 143.96, 132.22, 129.77 (Ar-overlap), 127.68 (Ar-overlap), 113.21, 107.61, 87.97, 62.40, 54.44, 49.41, 46.25, 45.78, 44.82, 41.24, 39.29, 37.43, 34.00, 31.04, 28.51, 28.20, 22.89, 21.48, 20.98, 12.35; HRMS m/z (ESI): calcd for C$_{28}$H$_{40}$N$_2$O$_5$SNa [M+Na]$^+$: 539.2550, found: 539.2549.

Cell viability assay
Cell viability was evaluated using an MTT assay. SW620 cell were seeded into 96-well plates at a density of $1.5 \times 10^4$ cells per well and stabilized at 37 °C for 24 h without CO$_2$. HCT116 cells and CaCo2 cells were seeded into 96-well plates at a density of $5 \times 10^3$ cells per well and stabilized at 37 °C for 24 h. Compounds were added to each well at various concentrations, and then the cells were incubated for 72 h. The MTT solution (10 μL 5 mg mL$^{-1}$) was added to each well, and the cells were incubated for another 4 h. Formazan crystals were dissolved in 150 μL of DMSO. Cell viability was assessed by measuring the absorbance at 490 nm wavelength using a microplate reader (BioTek ELx800 USA).

**Flow cytometric analysis of cell cycle arrest and apoptosis.**

SW620 cells were plated in 6-well plates (8x10$^5$ cells/well) and treated with different dose (0, 1, 10 and 20 μM) of 3l for 24 h. For cell cycle analysis, cells were washed with ice-cold PBS and fixed in 70% ethanol overnight at 4°C, and then stained with PI/RNase for 15 min at room temperature before analysis. To analyze the apoptotic rate, the treated cells were washed with ice-cold PBS twice and stained with 5 μl Annexin-V-FITC for 5 minutes and 5 μl 7AAD for another 10 minutes. After that, apoptosis was determined by flow cytometer (Guava Technologies; Merck KGaA, Darmstadt, Germany) and cell cycle distributions were analyzed by using ModFit software.

**Results And Discussion**

**Chemistry**

The synthetic route to the curcumol derivatives 3a-3m was outlined in Scheme 2. Firstly, chloroacetyl chloride was reacted with various secondary amines to produce intermediates 2a-2k in 70-90% yields, which was directly used into the next step without further purification. Then, the O-alkylation of curcumol with a-chloroacetamides 2a-2k in the presence of sodium hydride at room temperature to afford the desired products 3a-3k in 40-60% yields. In addition, removal of Boc group of compounds 3j and 3k gave the corresponding derivatives 3l and 3m in moderate yields with the treatment of TFA in DCM.

With the compound 3m in hand, we attempted to prepare a series of curcumol derivatives 4a-4g. As shown in Scheme 3, compound 3m was treated with alkyl chloride, aryl chloride, acyl chloride and sulfonyl chloride in the presence of t-BuOK to give the corresponding compounds 4a-4g in 60-90% yields, respectively.

**Cytotoxic activity and SAR analysis**

The antiproliferative evaluation of curcumol and its derivatives 3a-3m was initially carried out by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability and cytotoxicity assay in vitro. The resulting IC$_{50}$ values against three human CRC cell lines (SW620, HCT116 and CaCo2) were summarized in Table 1 with 5-fluorouracil (5-FU) as a positive control. As shown in Table 1, the results have revealed that most of the curcumol derivatives are more cytotoxic than curcumol against SW620, HCT116 and CaCo2 cells. The different amide substituents have the pronounced effects on anti-
proliferative activity. Thirteen compounds exhibited greater than 50% inhibition of SW620 cell growth, except for compounds 3b, 3c, 3k. Especially, many of the synthesized curcumol derivatives showed the good inhibitory activity against SW620 cells. Furthermore, the length of alkyl group on amide chain has affected on the antiproliferative activity. The IC$_{50}$ value of compound 3a containing dimethyl group is less than that of compound 3b and 3c bearing ethyl group and n-butyl group. Similarly, the antiproliferative activity is subjected to the ring size of cyclic amine. Compound 3d with 5-membered ring showed better activity, while compounds 3d and 3e with 5-membered ring, such as piperidinyl and homopiperidinyl ring, exhibited the low activity against the three CRC cell lines. In addition, compound 3g has the same activity as compound 3e. It is worthy to be noted that the IC$_{50}$ values of compounds 3l and 3m were significantly less than that of Boc-protected compounds 3j and 3k, respectively. Moreover, compound 3l exhibits the best antitumor activity, which was the most potent with the IC$_{50}$ value of 19.90 ± 0.64 μM (SW620 cell). Compound 3l is almost 5-fold more potent than curcumol and 2-fold more potent than 5-fluorouracil in SW620 cell. As well, compounds 3h and 3i bearing phenyl group showed good inhibitory activity, respectively. The results suggested that the introduction of phenyl group might have an advantageous effect on inhibition potency.

Table 1 Antiproliferative activities of curcumol and its derivatives against three human crc cell lines. a
<table>
<thead>
<tr>
<th>Compound</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM), 72 h, n ≥ 3</th>
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<tr>
<td></td>
<td>SW620</td>
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<tr>
<td>Curcumol</td>
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<tr>
<td>3a</td>
<td>29.90 ± 0.81</td>
</tr>
<tr>
<td>3b</td>
<td>50.74 ± 1.74</td>
</tr>
<tr>
<td>3c</td>
<td>59.66 ± 1.68</td>
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<tr>
<td>3d</td>
<td>27.22 ± 2.94</td>
</tr>
<tr>
<td>3e</td>
<td>28.04 ± 3.47</td>
</tr>
<tr>
<td>3f</td>
<td>46.78 ± 1.81</td>
</tr>
<tr>
<td>3g</td>
<td>22.62 ± 1.53</td>
</tr>
<tr>
<td>3h</td>
<td>24.24 ± 0.20</td>
</tr>
<tr>
<td>3i</td>
<td>29.94 ± 1.57</td>
</tr>
<tr>
<td>3j</td>
<td>43.04 ± 3.12</td>
</tr>
<tr>
<td>3k</td>
<td>60.88 ± 0.03</td>
</tr>
<tr>
<td>3l</td>
<td>19.90 ± 0.64</td>
</tr>
<tr>
<td>3m</td>
<td>35.84 ± 0.85</td>
</tr>
<tr>
<td>4a</td>
<td>29.74 ± 0.22</td>
</tr>
<tr>
<td>4b</td>
<td>21.06 ± 1.63</td>
</tr>
<tr>
<td>4c</td>
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</tr>
<tr>
<td>4d</td>
<td>22.42 ± 1.40</td>
</tr>
<tr>
<td>4e</td>
<td>100</td>
</tr>
<tr>
<td>4f</td>
<td>60.10 ± 2.40</td>
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<tr>
<td>4g</td>
<td>76.64 ± 4.80</td>
</tr>
<tr>
<td>5-FU</td>
<td>50</td>
</tr>
</tbody>
</table>

<sup>a</sup> MTT methods, cells were incubated with indicated compounds for 72 h, (means ± SD, n = 3).

Subsequently, the further modification of compound 3m was conducted to afford the corresponding compounds 4a-4g. The antiproliferative activities were evaluated and shown in Table 1. Compounds 4a-4d have stronger antitumor activity than 5-fluorouracil in SW620 cell line. Moreover, compounds 4a-4d have good selectivity to these cell lines. It was worthy to be noted that compound 4b displayed the better antiproliferative activity toward SW620 cells with an IC<sub>50</sub> value of 21.0 μM, which has nearly IC<sub>50</sub> value of...
compound 3l \((IC_{50} = 19.9 \, \mu M)\). Whereas, the selectivity of compound 4b toward this cell line substantially exceeded that of compound 3l.

The preliminary analysis of structure–activity relationships (SAR) suggested that the introduction of hydrophilic amide group and terminal free amino group at C-8 position of curcumol appeared crucial for an increase of the cytotoxicity. Furthermore, the less hindered steric substitutions, such as dimethyl group (3a) and pyrrolidinyl ring (3d), displayed more potent antitumor activity than those with bulky substitutions (3b-3c, 3f). Aromatic groups were favorable to enhance the inhibitory activity, such as compounds 3h and 3l. The acyl substituents on piperazinyl ring not only exhibited the better potent antitumor activity but also improved the selectivity toward the cell lines (compounds 4a-4d), whereas the introduction of N-sulfonyl groups rapidly reduced the antitumor activity (compounds 4f-4g).

**Cell apoptosis assay**

Apoptosis is an important biological process of programmed cell death, which maintains proper function and activity of cells. However, through evading apoptosis cells can proliferate continuously. Thus, utilizing chemical agents inducing apoptosis in cancer cells is a potent therapeutic strategy. To further investigate whether the cytotoxicity of 3l was linked to the induction of apoptosis, treated cells were analyzed using Annexin V-FITC/7AAD staining by flow cytometry. As shown in Fig. 3A and 3C, after treatment with 3l \((0 \, \mu M, 1 \, \mu M, 10 \, \mu M and 20 \, \mu M)\) for 24 h, the apoptosis rate of SW620 cells was markedly increased in a dose-dependent manner when compared with the control group (Fig. 2A, 2B). These results suggested that the induction of apoptosis can be a potential mechanism of the anticancer effect of 3l against CRC.

**Cell cycle analysis**

In order to detect whether 3l regulates the cell cycle of SW620 cells, cell cycle distribution was analyzed by flow cytometry. However, compared with the control group, the cell cycle distribution of SW620 cells was not significantly changed after administration with different concentrations of 3l \((0, 1, 10 and 20 \, \mu M)\) for 24 h (Fig. 2C, 2D). These results showed that the cytotoxicity of 3l has no connection with cell cycle arrest in SW620 cells.

**Conclusions**

In summary, a series of novel C-8 modified curcumol derivatives have been synthesized and tested for cytotoxicity against three cancer cell lines (SW620, HCT116 and CaCo2) by MTT assay in vitro. Within this series of curcumol derivatives, compounds 3a, 3d, 3g, 3h-3i, 3l-3m, and 4a-4d exhibited better anti-CRC cancer activity against human cancer SW620 cells, compared to positive controls such as 5-Fu and curcumol. The different amide functional groups of curcumol derivatives had pronounced effects on anti-proliferative activity. Among them, curcumol derivative 3l was the most promising derivative. The substitution of (hetero)aryl group on piperazinyl ring tended to increase the anticancer activity, and have better inhibitory effect than curcumol. The preliminary SAR of the target compounds was discussed...
based on the experimental data obtained. Further studies on the structure modification of curcumol and the mechanism of the derivatives are currently in progress and will be reported in due course.

**Declarations**

**Conflicts of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**References**

Figures

Curcumol

Englerin A

Arglabin

Figure 1

The chemical structure of curcumol, englerin A and arglabin.
Figure 2

(A) Analysis of SW620 cell apoptosis induced by 3l after 24 h drug exposure; (B) Quantitative detection of 3l induced apoptosis; (C) The cell-cycle distributions were determined in CRC cells exposed with 3l after 24 h drug exposure by staining with PI solution; (D) Quantitative detection of 3l induced cell-cycle progression.
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